



1/7

PHOSPHODIESTER BOND AT 5' SIDE OF AP SITE

PHOSPHODIESTER BOND AT 3' SIDE OF AP SITE

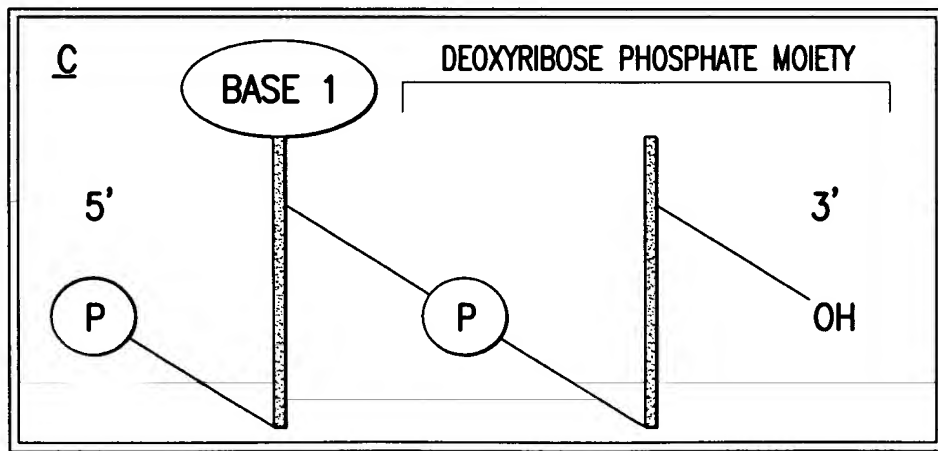
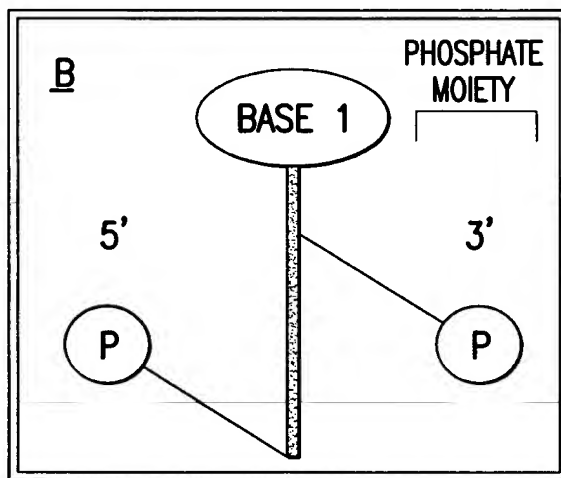
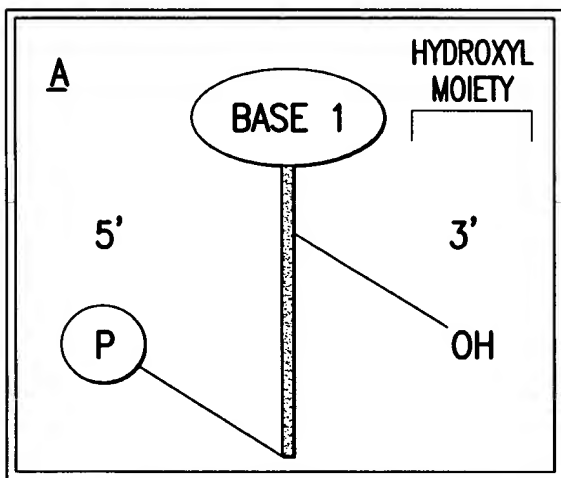
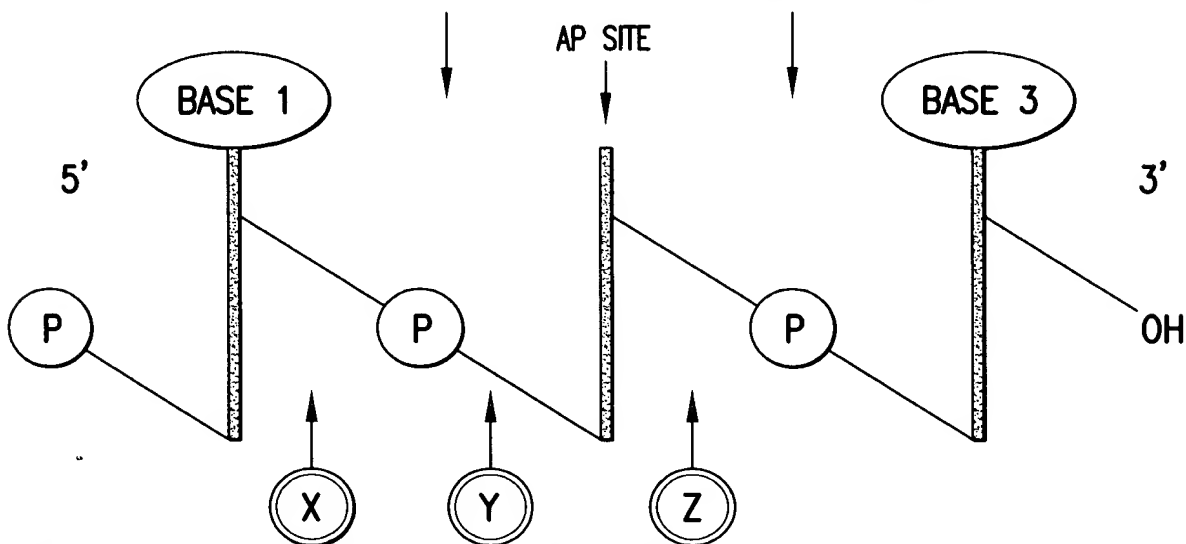


FIG.1

2/7

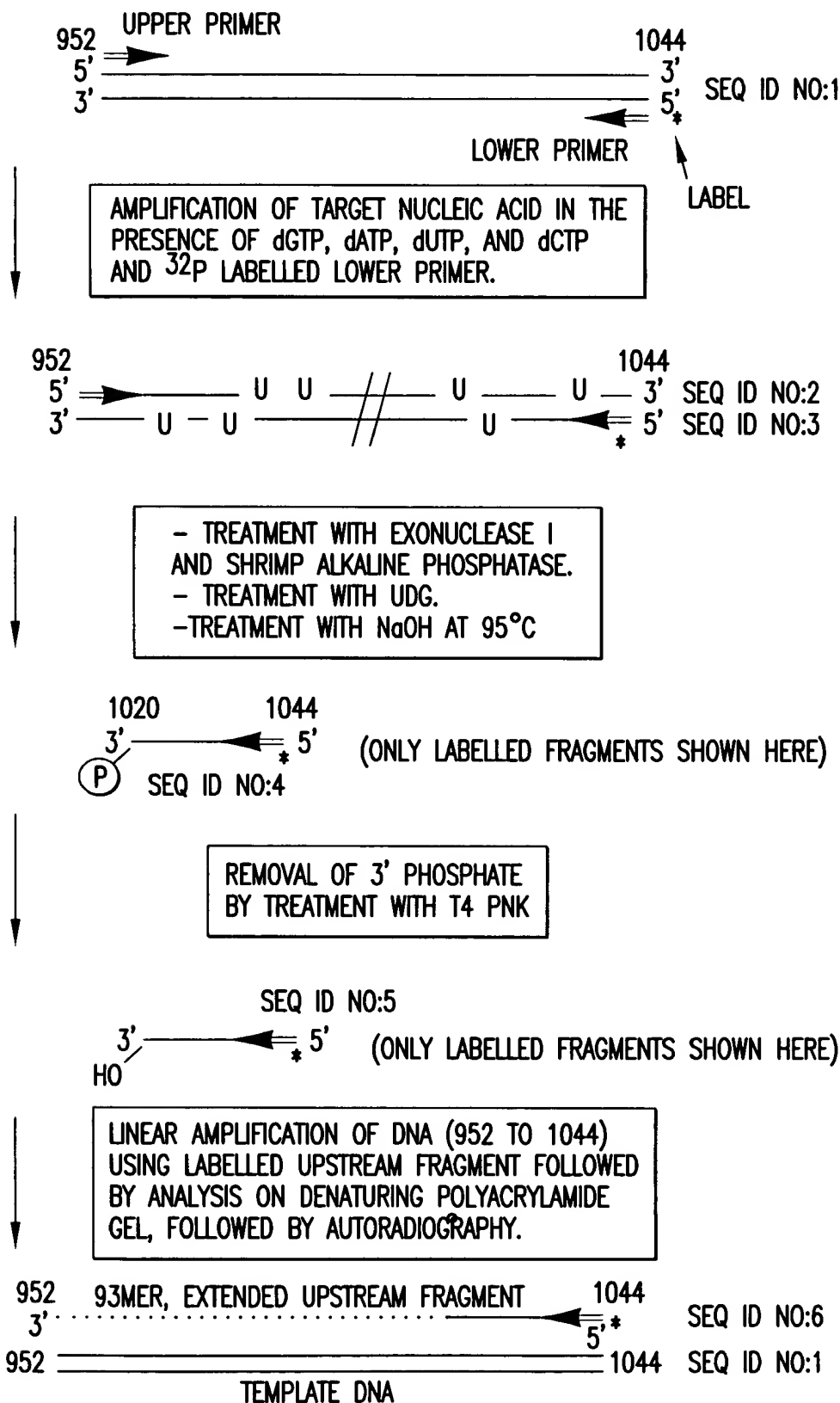
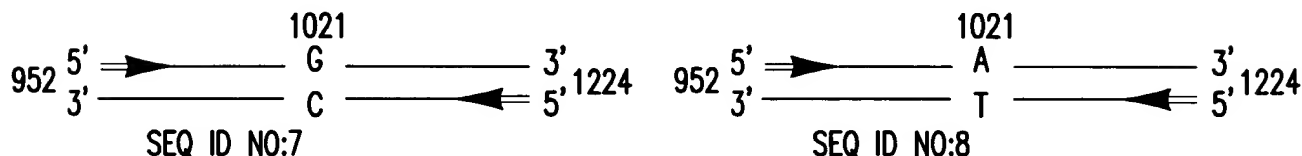


FIG.2

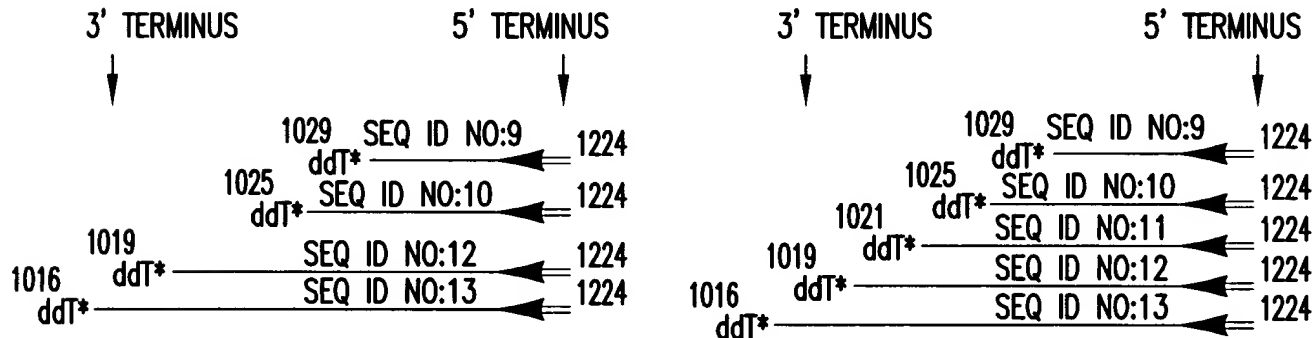
3/7

– AMPLIFICATION OF NORMAL AND MUTANT TARGET NUCLEIC ACID IN THE PRESENCE OF dGTP, dATP, dCTP AND 1/20 RATIO OF ddTTP TO dTTP.



– TREATMENT WITH EXONUCLEASE I AND SHRIMP ALKALINE PHOSPHATE.
– TREATMENT WITH UDG.
– TREATMENT WITH NaOH AT 95°C
– DNA IS PRECIPITATED.
– TREATMENT WITH T4 PNK

EXTENSION OF THE UPSTREAM FRAGMENTS GENERATED ABOVE IN THE PRESENCE OF ³³P-LABELLED ddTTP* AND UNLABELLED ddGTP, ddATP, AND ddCTP.



ONLY SOME FRAGMENTS CORRESPONDING TO CLEAVAGE AT U INCORPORATION SITES SURROUNDING THE MUTATION SITE ARE SHOWN HERE.

DETECTION OF EXTENDED LABELLED FRAGMENTS BY PAGE AND AUTORADIOGRAPHY

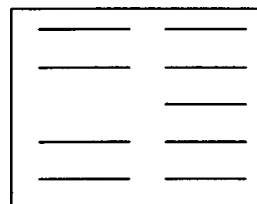
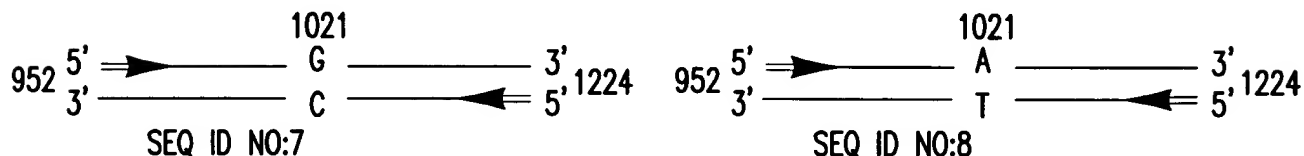


FIG.3

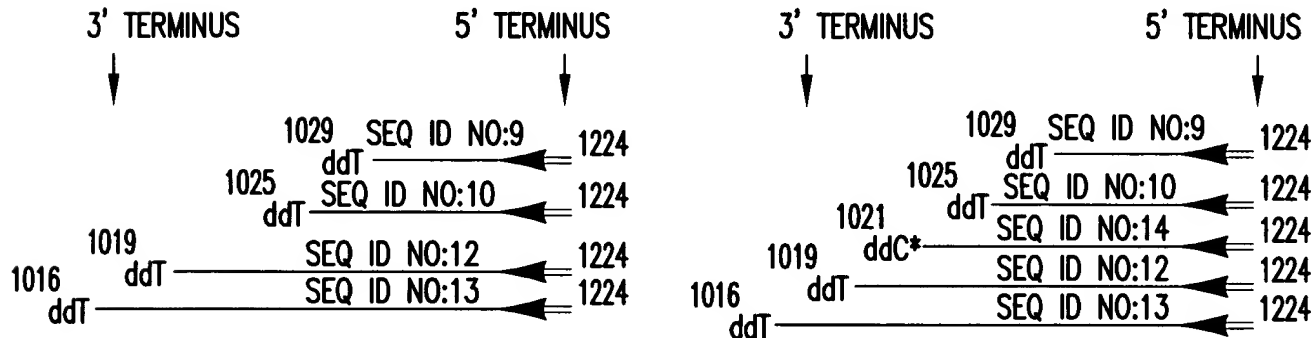
4/7

- AMPLIFICATION OF NORMAL AND MUTANT TARGET NUCLEIC ACID IN THE PRESENCE OF dGTP, dATP, dCTP AND 1/20 RATIO OF dUTP TO dTTP.

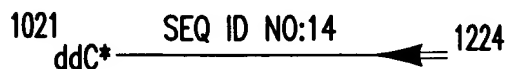


- TREATMENT WITH EXONUCLEASE I AND SHRIMP ALKALINE PHOSPHATE.
- TREATMENT WITH UDG.
- TREATMENT WITH NaOH AT 95°C
- DNA IS PRECIPITATED.
- TREATMENT WITH T4 PNK

EXTENSION OF THE UPSTREAM FRAGMENTS GENERATED ABOVE IN THE PRESENCE OF ^{33}P -LABELLED ddCTP* AND UNLABELLED ddGTP, ddATP, AND ddTTP.



ONLY SOME FRAGMENTS CORRESPONDING TO CLEAVAGE AT U INCORPORATION SITES SURROUNDING THE MUTATION SITE ARE SHOWN HERE.



DETECTION OF EXTENDED LABELLED FRAGMENTS BY PAGE AND AUTORADIOGRAPHY

FIG.4

5/7

MUTATION SITE

A

UPPER PRIMER SEQ ID NO:15

6390 6443

5' AACTTGTGGTAGTTGGAGCT GGTGGCGTAGGCAAGAGTGCC TTGACGATACAGC 3'

3' TTGAACACC ATCAA CCTCG ACCAC CGCAT CC GTTC TCA CG GA ACTGC TATGT CG 5'

T

LOWER PRIMER

AMPLIFICATION OF TARGET NUCLEIC ACID IN THE PRESENCE OF dGTP, dATP, dUTP, AND dCTP.

SEQ ID NO:16
 5' AACTTGTGGTAGTTGGAGCTGGUGGCGUAGGCAAGAGUGCCUUGACGAUACAGC 3'
 3' UUGAACACCAUCAACCUCGACUACCGCATCCGTTCTCACGGAAGTGTATGTCG 5'
 SEQ ID NO:17

SEQ ID NO:18

AMPLIFIED MUTANT ALLELE

5' AACTTGTGGTAGTTGGAGCTGAUGGCGUAGGCAAGAGUGCCUUGACGAUACAGC 3'

3' UUGAACACCAUCAACCUCGACCACCGCATCCGTTCTCACGGAAGTGTCTATGTCG 5'

SEQ ID NO:19

- TREATMENT WITH EXONUCLEASE I AND SHRIMP ALKALINE PHOSPHATASE.
- TREATMENT WITH UDG.
- TREATMENT WITH ENDO IV.

SEQ ID NO:20 NORMAL UPSTREAM FRAGMENT
3' CGACCACCGCATCCGTTCTCACGGAAGTCTATGTCG 5'

SEQ ID NO:21 MUTANT UPSTREAM FRAGMENT
3' ACCGCATCCGTTCTCACGGAAGTCTATGTCG 5'

FIG.5

6/7

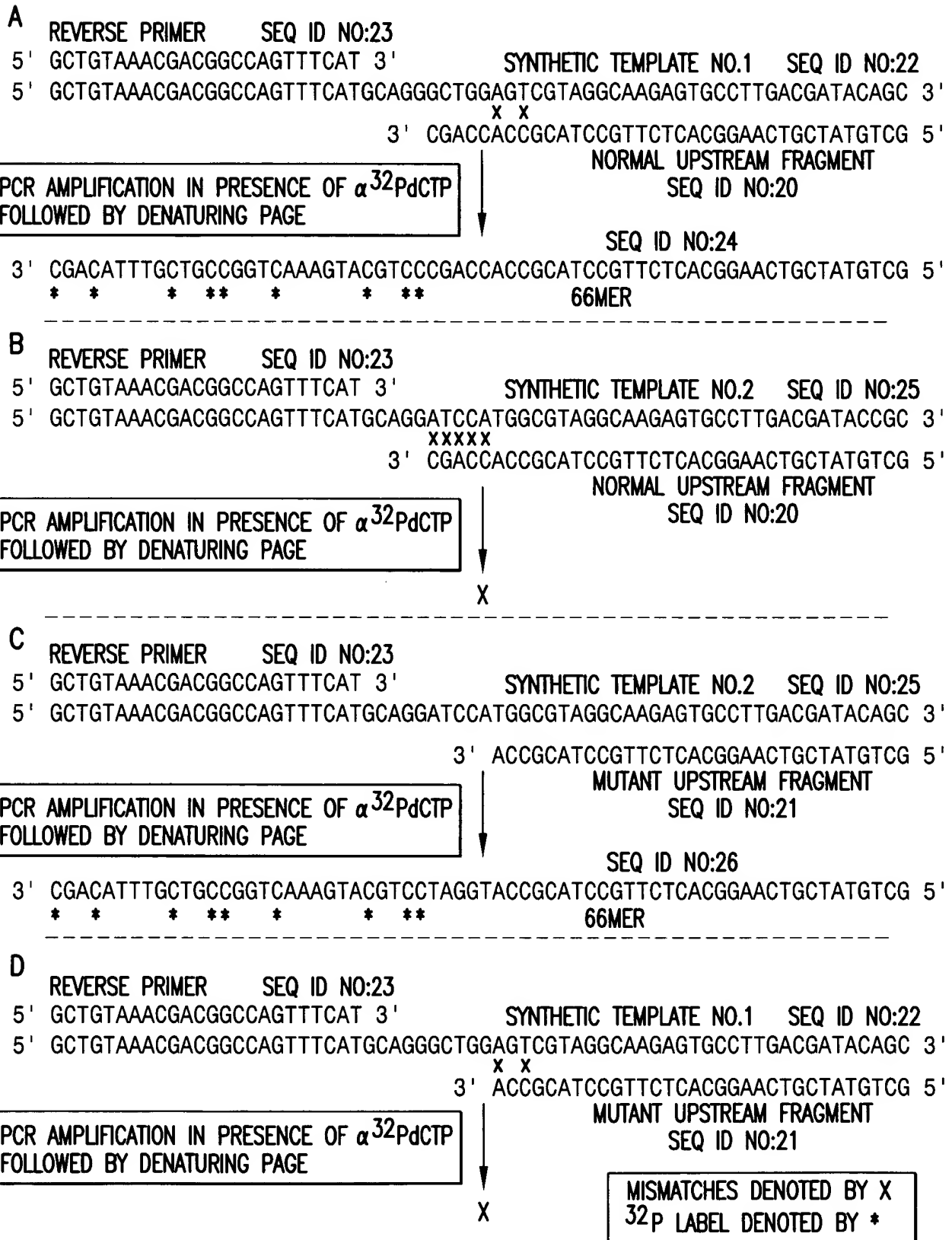


FIG.6

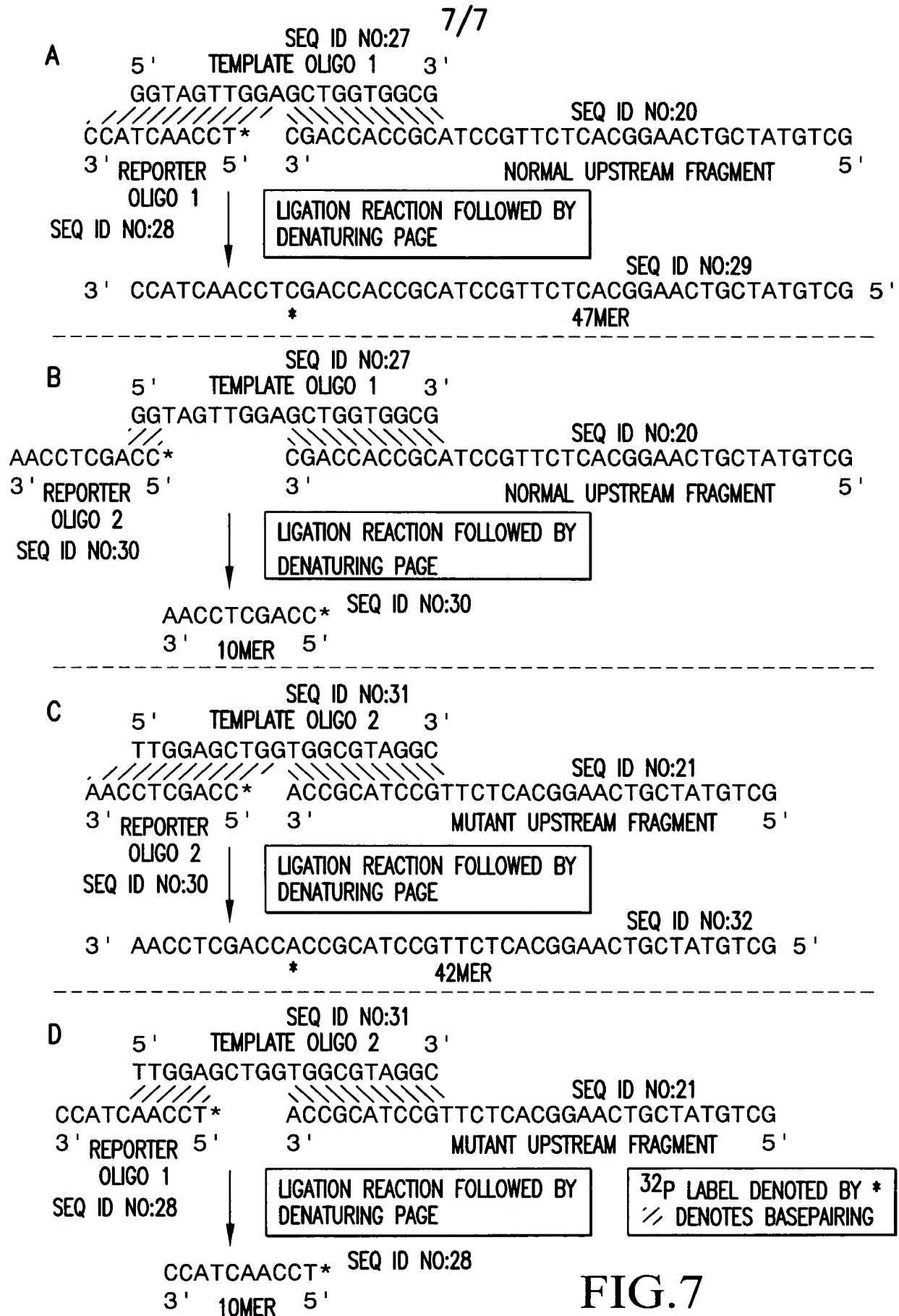


FIG.7